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INSTRUCTIONS FOR AVIAN SWAB AND BLOOD COLLECTION AND SUBMISSION FOR MOLECULAR DIAGNOSTIC TESTING

I. General Sample Considerations (see each test for additional instructions)

IF SNAP-CAP TUBES ARE USED FOR BLOOD AND/OR SERUM, THEY MUST BE SEALED WITH PARAFILM FOR SHIPPING. IF SENDING BLOOD/SERUM FROM MORE THAN ONE ANIMAL, PLEASE PLACE EACH ANIMAL'S BLOOD/ SERUM IN A SEPARATE ZIPLOCK BAG TO REDUCE CROSS-CONTAMINATION.

A. Whole Blood:

Approximately 0.1 ml of whole, unclotted blood obtained by vena puncture is requested (note: the same blood sample can be used for any tests requiring whole blood). EDTA (purple top) or heparin (green top) can be used as an anticoagulant. It is important that the ratio of blood to anticoagulant is correct and we recommend 1.3 ml screw cap tubes by Sarstedt or equivalent. An improper ratio of blood to anticoagulant can cause a false negative result. Hemolysis can also affect test accuracy.

SWAB SAMPLES IN GENERAL: A sterile culturette should be used to collect the samples described below. The culturette should be placed back in its plastic sheath prior to shipping. The transport media ampoule may or may not be activated. We recommend culturettes manufactured by Becton Dickinson or equivalent for sample collection. If you choose a different culturette it **must not contain a gel based transport media**. Gel based transport media may interfere with nucleic acid processing, and potentially cause a false negative result.

B. Swab of excrement

A sterile culturette should be used to collect a sample from the cloaca or fresh feces (note: the same culturette can be used for performing any test requiring this sample type). **A properly collected swab should be completely coated with excrement**. If the swab is not coated with excrement, then the sample may be insufficient to detect small quantities of target nucleic acid. When fresh feces are used in lieu of a cloacal sample, one should be aware that the sample may be contaminated with viral nucleic acid after it is excreted from the bird.

C. Swab of tissues

A sterile culturette (note: the same culturette can be used for performing any test requiring this sample type) should be used to collect a sample from the tissue of interest. Collect sample aseptically. Overnight shipping with ice packs is highly recommended. Do not freeze.

D. Swab of bird's living area

A sterile culturette should be used to collect a sample from the test environment (note: the same culturette can be used for performing all assays requiring this sample). Aggressive rubbing of multiple areas where feces or fecal dust accumulates (*e.g.* corners, ceiling fans, picture frames, air filters) will provide the best sample and increase the likelihood of finding small quantities of nucleic acid.

E. Serum or Plasma

Approximately 0.1 to 0.15 ml of serum or plasma is requested (note: the same serum or plasma sample can be used for performing any test requiring serum or plasma). **The serum or plasma should be separated, spun down and the clear serum or plasma transferred to a new, sterile tube before shipment**. Hemolyzed samples can give false results and if the hemolysis is too extensive tests cannot be run.

F. Feathers

Several affected blood feathers, associated follicles, and surrounding skin placed in 10% buffered formalin.

II. Testing for Psittacine Circovirus (PCV 1 & PCV 2) Psittacine Beak & Feather Disease Virus

For detecting Psittacine Beak and Feather Disease Virus, which is a circovirus, you can now choose whether to test a bird for psittacine circovirus 1 (PCV 1), psittacine circovirus 2 (PCV 2) or both. To date, we have found psittacine circovirus 2 as the primary variant present in lories and lorikeets. The Emerging Diseases Research Group has documented that some lories with PCV 2 associated disease can recover. We have documented mixed PCV 1 and PCV 2 infections in other psittacines but have yet to determine what effect, if any, the mixed infection has on prognosis.

Until a safe subunit vaccine is available, testing of birds for the presence of circoviral nucleic acid followed by the isolation of persistently infected birds is the best method to reduce the spread of this virus among Psittaciformes. The PCV (formerly PBFD) virus DNA detection assay* uses viral specific nucleic acid primers and a probe to detect as few as 10 copies of a small segment of viral specific DNA in white blood cells, swabs of feather pulp or swabs of samples collected from a bird's living area that may be contaminated with viral nucleic acid.[1,2] The test can be used to detect target nucleic acid in the blood of birds with active disease (feather abnormalities are discernible), birds who are in the process of clearing an infection (nucleic acid is present in white blood cells in the absence of feather abnormalities) and in birds who are actively infected but have not yet developed obvious feather abnormalities.

As a screening test for birds with normal feathers, we recommend testing whole blood for the presence of the target segment of circoviral nucleic acid.

If a bird has abnormally developed or developing feathers, we recommend a feather pathology panel. This panel includes evaluation of whole blood for the presence of circoviral nucleic acid and histologic examination of an affected feather and surrounding skin. This combination of tests will determine if feather abnormalities are caused by psittacine circovirus, and if not, will help identify the type of microscopic lesions associated with the feather abnormalities, which might provide insight into an etiology. Additional ancillary testing, special stains for bacteria or fungi, *in situ* hybridization for PCV virus, polyomavirus or adenovirus may be performed as determined necessary by the attending pathologist.

Required samples: Blood (see **A**) or Environmental swab (see **D**)
Feather Pathology Panel: Blood (see **A**) and formalin-fixed abnormal blood feathers, their follicles and surrounding skin (see **F**)

III. Testing for Polyomavirus

As is the case with many viral-induced diseases in companion animals, the most effective way to control the continued spread of polyomavirus is through vaccination.[a,3,4] When necessary, DNA probe testing* can be used to detect as few as 10 copies of a small segment of polyomaviral specific DNA in excrement, tissues or swabs of samples collected from a bird's living area that may be contaminated with polyomaviral nucleic acid. The clinician can choose either a DNA probe assay for detection of target nucleic acid in excrement (or environmental swab) or can choose a polyomavirus panel that includes several DNA probe assays and serology.

Testing for the presence of a target segment of polyomavirus nucleic acid can be used to determine if a bird is shedding the virus in excrement or if a sampled environment has been contaminated with polyomavirus. An antibody assay can be used to determine if a bird has been previously infected with the virus (the speed of decay of

detectable levels of antibodies varies with the individual bird) or has developed a measurable antibody response following vaccination. Repeat sampling can be used to confirm an acute infection.

If one chooses to use any diagnostic test for polyomavirus, it is important to ask what question is to be answered. If the question is, "Has a bird been previously infected with polyomavirus?", then the test that is likely to provide the best information is an antibody assay. Antibody titers will persist long past the time that a bird has cleared an infection. If the question is, "Does a bird present an immediate threat to other birds in a group?", then the best test to run is a DNA probe assay on excrement. Birds which are shedding polyomavirus nucleic acid can be separated from other birds until they are able to clear the infection, and during the time that the remainder of the flock is responding to vaccination.[2,4]
If testing is chosen for attempted control of polyomavirus (note: vaccination is the best method to reduce the continued spread of polyomavirus among Psittaciformes), we recommend a polyomavirus panel that includes tests to demonstrate if polyomaviral nucleic acid can be detected in the excrement, or blood, and if virus-neutralizing antibodies are present in serum. For postmortem diagnosis of polyomavirus, we recommend histopathology through the Zoo and Exotic Animal Service (ZEAPS), and *in situ* hybridization.

Required Samples: Choana / Cloaca Swab (see **B**) or Environmental Swab (see **D**)
Polyomavirus Panel: Blood (see **A**)**,** Swab (see **B**) and Serum (see **E**)

IV. Testing for Chlamydophila spp.

Until a safe subunit vaccine is available, testing of birds for chlamydial infections followed by isolation and appropriate therapy is a method that may help reduce the spread of this organism among companion birds.[5,6] To facilitate control of this organism, the clinician can choose either a DNA probe assay for detection of target nucleic acid in excrement (or environmental swab) or can choose a Chlamydophila panel that includes several DNA probe assays and serology. The Chlamydophila DNA detection assay* detects a small segment of Chlamydophila nucleic acid in white blood cells, tissues, excrement or swabs of samples collected from a bird's living area that may be contaminated with Chlamydophila nucleic acid.

Testing for the presence of a target segment of Chlamydophila nucleic acid can be used to determine if a bird is shedding chlamydial nucleic acid in excrement or if a sampled environment has been contaminated with Chlamydophila. An antibody assay can be used to determine if a bird has been previously infected with the organism (the speed of decay of detectable levels of antibodies varies with the individual bird). Repeat sampling can be used to confirm an acute infection.

When testing for Chlamydophila, it is important to ask what question is to be answered. If the question is, "Has a bird been previously infected clinically or sub clinically with Chlamydophila?", then the test that is likely to provide the best information is an antibody assay. Antibody titers will persist long past the time that a bird has recovered from a Chlamydophila infection. If the question is, "Does a bird present an immediate threat to other birds in a group?", then the best test to run is a DNA probe assay on a choanal / cloacal swab. Birds which are shedding chlamydial nucleic acid can be separated from other birds until they are able to clear the infection. Testing of excrement for the presence of chlamydial nucleic acid will provide clinically relevant information with respect to immediate management of a patient. Detecting that a bird is actively passing chlamydial nucleic acid in its excrement facilitates isolation of the bird while it is being treated.

As a screening assay, we recommend a Chlamydophila panel that includes tests to demonstrate if chlamydial nucleic acid can be detected in a choanal / cloacal swab, or blood, and if Chlamydophila-specific antibodies are present in serum. Chlamydophila serology is performed in conjunction with the Avian and Wildlife Laboratory, Division of Comparative Pathology at the University of Miami School Of Medicine.

For postmortem diagnosis of chlamydiosis we currently recommend PCR of swabs from liver, choana/cloaca, histopathology through ZEAPS.

Required Samples: Choanal/Cloacal Swab (see **C**) or Environmental Swab (see **D**)
Chlamydophila Panel: Choanal/Cloacal Swab (see **C**), Blood (see **A)** and Serum (see **E**)

IV. **Sex Identification for most Psittacine Birds**

We recommend that DNA probe technology be used for sex identification only in companion birds. Any bird intended for breeding purposes, or with reproductive problems, should be evaluated by an experienced endoscopist and through chromosomal analysis**.

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*The PCV, polyomavirus, and gender DNA detection assays are based on tests developed by the Psittacine Disease Research Group at the University of Georgia's College of Veterinary Medicine. The Chlamydophila DNA detection assays are based on Chlamydophila nucleic acid tests developed by the Psittacine Disease Research Group and researchers at the Louisiana State University's College of Veterinary Medicine. These assays are used by the Infectious Diseases Laboratory through license agreements with the University of Georgia Research Foundation and Hoffmann La Roche. Known positive and known negative control samples are run with each group of samples to insure test accuracy and monitor for inadvertent contamination of test samples within the laboratory.

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[a]Polyomavirus Vaccine; Biomune, Lenexa, KS. (913) 894-0230

[1] Niagro, F.N., *et.al.*: Proc. Assoc. Avian Vet, 1990; 25-37.
[2] Ritchie, B.W. Avian Viruses: Function and Control. Wingers Publishing; 1995.
[3] Ritchie, B.W., *et.al.*: Am J Vet Res 1998; 59: 143-148.
[4] Ritchie, *et.al.*: J Am Vet Med Assoc 1998; 212:1-6.
[5] Gerlach H. Chlamydia, In Avian Medicine: Principles and Application; Wingers Publishing; 1994; 984-996.
[6] Tully, T.N., *et.al.*: Proc. Assoc. Avian Vet. 1996; 161-162.